AMENDMENTS

Amendments to the Specification

Please replace the paragraph on page 1, at indicated lines 1 – 3 with the following paragraph:

Plants expressing Δ6-desaturase genes, ~~PUFAS-containing [sic]~~ PUFAs-containing oils from these plants, and a process for the preparation of unsaturated fatty acids

Please replace the paragraph on page 1, at indicated lines 6 – 13 with the following paragraph:

The present invention relates to an improved process for the preparation of unsaturated fatty acids and to a process for the preparation of triglycerides with an increased content of unsaturated fatty acids. The invention relates to the generation of a transgenic organism, preferably of a transgenic plant or of a transgenic microorganism, with an increased content of fatty acids, oils or lipids with Δ6 double bonds owing to the expression of a moss ~~Δ-6-desaturase [sic]~~ Δ6-desaturase.

Please replace the paragraph on page 2, at indicated lines 1 – 43 with the following paragraph:

Owing to their beneficial properties, there has been no lack of attempts in the past to make available genes which are involved in the synthesis of fatty acids or triglycerides for the production of oils in various organisms with a modified content of unsaturated fatty acids. Thus, a Δ9-desaturase is described in WO 91/13972

and its US equivalent. WO 93/11245 claims a Δ15-desaturase, while WO 94/11516 claims a Δ12-desaturase. Δ6-desaturases are described in Girke et al. (The Plant Journal, 15, 1998: 39-48), Napier et al. (Biochem. J., 330, 1998: 611-614), Murata et al. (Biosynthesis of γ-linolenic acid in cyanobacterium Spirulina patensis, pp. 22-32, In: γ-linolenic acid, metabolism and its roles in nutrition and medicine, Huang, Y. and Milles, D.E. [eds.], AOC Press, Champaign, Illinois), Sayanova et al. (Proc. Natl. Acad. Sci. USA, 94, 1997: 4211-4216), WO 98/46764, Cho et al. (J. Biol. Chem., 274, 1999: 471-477), Aki et al. (Biochem. Biophys. Res. Commun., 255, 1999: 575-579), and Reddy et al. (Plant Mol. Biol., 27, 1993: 293-300). Further desaturases are described, for example, in EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-0 794 250, Stukey et al., J. Biol. Chem., 265, 1990: 20144-20149, Wada et al., Nature 347, 1990: 200-203 or Huang et al., Lipids 34, 1999: 649-659. Further Δ6-desaturase are described in WO 93/06712, US 5,614,393, US 5,614,393, WO 96/21022, WO 00/21557 and WO 99/27111. The biochemical characterization of the various desaturases is, however, inadequate as yet because the enzymes, being membrane-bound proteins, can be isolated and characterized only with great difficulty (McKeon et al., Methods in Enzymol. 71, 1981: 12141-12147, Wang et al., Plant Physiol. Biochem., 26, 1988: 777-792). As a rule, membrane-bound desaturases are characterized by introducing them into a suitable organism which is subsequently tested for enzyme activity by analyzing the starting material and the product. The use for production in transgenic organisms described as in WO 98/46763 WO 98/46764, WO 98/46765. The expression of various desaturases as in WO 99/64616 or WO 98/46776 and the formation of polyunsaturated fatty acids is also described and claimed here. As regards the expression efficacy of desaturases and their effect on the formation

of polyunsaturated fatty acids, it must be noted that expression of an individual desaturase as described in the above prior art only led to, and leads to, low contents of unsaturated fatty acids, for example ~~Δ-6-unsaturated [sic]~~ Δ6-unsaturated fatty acids/lipids such as, for example, γ-linoleic acid, being achieved.

Please replace the paragraph on page 3, at indicated lines 11 – 32 with the following paragraph:

We have found that this object is achieved by a process for the preparation of unsaturated fatty acids, which comprises introducing, into an organism, at least one isolated nucleic acid sequence encoding a polypeptide having Δ6-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence having the sequence shown in SEQ ID NO: 1,

b) nucleic acid sequences which, as a result of the degeneracy of the genetic code, are derived from ~~[lacuna]~~ the sequence shown in SEQ ID NO: 1,

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 1 which encode polypeptides with the amino acid sequences shown in SEQ ID NO: 2 and have at least 50% homology at the amino acid level without substantially reducing the enzymatic action of the polypeptides,

and culturing this organism, where the cultured organism contains at least 1 mol% of unsaturated fatty acids based on the total fatty acid content in the organism.

Please replace the paragraph on page 3, indicated line 39 – page 4, indicated line 21 with the following paragraph:

The organisms obtained by the processes according to the invention contain, as a rule, unsaturated fatty acids in the form of bound fatty acids, i.e. the unsaturated fatty acids exist predominantly in the form of their mono-, di- or triglycerides, glycolipids, lipoproteins or phospholipids such as oils or lipids or else as fatty acids bound as esters or amides. Free fatty acids are also present in the organisms in the form of the free fatty acids or in the form of their salts. Advantageously, the free or bound unsaturated fatty acids have an increased content of fatty acids with Δ6 double bonds, such as, advantageously, γ-linoleic acid, which is increased over that of the starting organisms. The organisms obtained by culturing in the process according to the invention, and the unsaturated fatty acids which they contain, can be used directly, for example for the production of pharmaceutical products, of agrochemicals, feeds or foodstuffs or else after isolation from the organisms. All steps of the purification of the unsaturated fatty acids can be used, that is to say that ~~[lacuna]~~ all steps from crude extracts of the fatty acids up to fully purified fatty acids are suitable for preparing the abovementioned products. In an advantageous embodiment, the bound fatty acids can be liberated from the, for example, oils or lipids for example by hydrolysis with bases, such as, for example, with NaOH or KOH. These free fatty acids can be used directly in the mixture obtained or after further purification for producing pharmaceutical products, agrochemicals, feeds of foodstuffs. Also, the bound or free fatty acids can be used for transesterification or esterification, for example with other mono, di- or triglycerides or glycerol in order to increase the content of unsaturated fatty acids in these

compounds, for example in the triglycerides.

Please replace the paragraph on page 6, at indicated lines 8 – 14 with the following paragraph:

The invention furthermore relates to unsaturated fatty acids and to ~~trigylcerides [sic]~~ triglycerides with an increased content of unsaturated fatty acids which have been prepared by the abovementioned methods, and to their use for the production of foodstuffs, feeds, cosmetics or pharmaceuticals. To this end, they are added in customary quantities to the foodstuffs, the feeds, the cosmetics or the pharmaceuticals.

Please replace the paragraph on page 6, at indicated lines 16 – 30 with the following paragraph:

In the process according to the expression, higher contents of unsaturated fatty acids such as γ-linolenic acid were obtained by expressing a moss Δ6-desaturase in organisms such as fungi, bacteria, animal or plants, preferably fungi, bacteria and plants, especially preferably in plants, very especially preferably in oil crops such as oilseed rape, canola, linseed, soybean, sunflower, borage, castor, oilpalm, safflower (Carthamus tinctorius), coconut, peanut or cacao bean. Expression in field crops such as maize, wheat, rye, oats, triticale, rice, barley, alfalfa or bush plants (coffee, cacao, tea) is also advantageous. Expression in the abovementioned organisms of a gene which encodes a moss ~~Δ-6-desaturase [sic]~~ Δ6-desaturase allows contents of unsaturated fatty acids of at least 1 mol%, preferably at least 3 mol%, especially preferably at least 4 mol%, very especially preferably at least 5 mol%, to be

achieved in the organisms.

Please replace the paragraph on page 7, at indicated lines 12 – 14 with the following paragraph:

The derivatives mentioned can be isolated, for example, from other organisms ~~[lacuna]~~, for example eukaryotic organisms such as plants, such as, especially, mosses, dinoflagellates or fungi.

Please replace the paragraph on page 8, at indicated lines 14 – 41 with the following paragraph:

Standard conditions mean, for example, depending on the nucleic acid, temperatures between 42 and 58°C in an aqueous buffer solution with a concentration between 0.1 ~~to [sic]~~ and 5 x SSC (1 X SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.2) or additionally in the presence of 50% formamide, such as, for example, 42°C in 5 x SSC, 50% formamide. The hybridization conditions for DNA:DNA hybrids are advantageously 0.1 x SSC and temperatures between about 20°C ~~to [sic]~~ and 45°C, preferably between about 30°C ~~to [sic]~~ and 45°C. The hybridization conditions for DNA:RNA hybrids are advantageously 0.1 x SSC and temperatures between about 30°C ~~to [sic]~~ and 55°C, preferably between about 45°C ~~to [sic]~~ and 55°C. These temperatures stated for the hybridization are melting temperatures calculated by way of example for a nucleic acid with a length of about 100 nucleotides and a G + C content of 50% in the absence of formamide. The experimental conditions for DNA hybridization are described in relevant textbooks of genetics such as, for example, Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989, and can be calculated by the formulae known to the skilled worker, for

example depending on the length of the nucleic acids, the nature of the hybrids or the G + C content. Further information on hybridization can be found by the skilled worker in the following textbooks: Ausubel et al. (eds), 1985, Current Protocols in Molecular Biology, John Wiley & Sons, New York; Hames and Higgins (eds), 1985, Nucleic Acids Hybridization: A Practical Approach, IRL Press at Oxford University Press, Oxford; Brown (ed), 1991, Essential Molecular Biology: A Practical Approach, IRL Press at Oxford University Press, Oxford.

Please replace the paragraph on page 13, indicated line 1 – page 14, indicated line 11 with the following paragraph:

Suitable promoters in the expression cassette are, in principle, all promoters which are capable of controlling the expression of foreign genes in organisms, advantageously in plants of fungi. It is preferable to use in particular a plant promoter or promoters derived from, for example, a plant virus. Examples of advantageous regulatory sequences for the process according to the invention are present, for example, in promoters such as the cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, $lacI^{q}$, T7, T5, T3, gal, trc, ara, SP6, λ-P_R or in the λ-P_L promoter which are advantageously used in Gram-negative bacteria. Further advantageous regulatory sequences are present, for example, in the Gram-positive promoters amy and SPO2, in the yeast or fungal promoters ADC1, MFa, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH or in the plant promoters such as CaMV/35S [Franck et al., Cell 21(1980) 285-294], RUBISCO SSU, OCS, B33, nos (= Nopaline Synthase Promoter) or in ubiquitin promoter. The expression cassette can also comprise a chemically inducible promoter by which expression of the exogenous Δ6-desaturase gene in the organisms,

advantageously in the plants, can be controlled at a particular time. Examples of such advantageous plant promoters are the PRP1 promoter [Ward et al., Plant. Mol. Biol. 22 (1993), 361-366], a benzenesulfonamide-inducible promoter (EP 388186), a tetracycline-inducible promoter (Gatz et al., (1992) Plant J. 2,397-404), a salicylic acid-inducible promoter (WO 95/19443), an abscisic acid-inducible promoter (EP335528) and an ethanol- or cyclohexanone-inducible promoter (WO 93/21334). Further plant promoters are, for example, the potato cytosolic FBPase promoter, the potato ST-LSI promoter (Stockhaus et al., EMBO J. 8 (1989) 2445-245), the Glycine max phosphoribosyl-pyrophosphate amidotransferase promoter (see also Genbank Accession Number U87999) or a node-specific promoter ~~in EP 249676 as can be advantageously used [sic]~~ as are advantageously used in EP 249676. Particularly advantageous plant promoters are those which ensure expression in tissues or plant parts/organs in which fatty acid biosynthesis or its precursors take place, such as, for example, in the endosperm or the developing embryo. Particular mention should be made of advantageous promoters which ensure seed-specific expression, such as, for example, the USP promoter or derivatives thereof, the LEB4 promoter, the phaseolin promoter or the napin promoter. The USP promoter which has been stated in accordance with the invention and which is particularly advantageous, or its derivatives, mediate very early gene expression during seed development (Baeumlein et al., Mol Gen Genet, 1991, 225 (3): 459-67). Other advantageous seed-specific promoters which can be used for monocotyledonous and dicotyledonous plants are the promoters suitable for dicots such as, for example, the oilseed rape napin gene promoter (US5,608,152), the Arabidopsis oleosin promoter (WO98/45461), the Phaseolus vulgaris phaseolin promoter

(US5,504,200), the Brassica Bce4 promoter (WO91/13980) or the legume B4 promoter (LeB4, Baeumlein et al., Plant J., 2, 2, 1992: 233 - 239) or promoters which are suitable for monocots, such as the barley lpt2- or lpt1-gene promoters (WO95/15389 and WO95/23230) or the promoters of the barley hordein gene, of the rice glutelin gene, of the rice oryzin gene, of the rice prolamin gene, of the wheat gliadin gene, of the wheat glutelin gene, of the maize zein gene, of the oat glutelin gene, of the sorghum kasirin gene or of the rye secalin gene, which are described in WO99/16890.

Please replace the paragraph on page 16, at indicated lines 12 – 19 with the following paragraph:

The DNA sequence encoding a ~~Phsycomitrella [sic]~~ Physcomitrella patens Δ6-desaturase comprises all sequence characteristics which are necessary to achieve correct localization for the site of fatty acid, lipid or oil biosynthesis. No further targeting sequences are therefore necessary per se. However, such localization may be desirable and advantageous and can therefore be modified or enhanced artificially, so that such fusion constructs are also a preferred advantageous embodiment of the invention.

Please replace the paragraph on page 16, indicated line 30 – page 17, indicated line 1 with the following paragraph:

The nucleic acid sequences encoding Δ6-desaturase genes are advantageously cloned together with at least one reporter gene into an expression cassette which is introduced into the organism via a vector or directly into the genome. This reporter gene should make easy detection possible by a growth, fluorescence, chemo- or bioluminescence or resistance assay or by a photometric measurement. Examples

of reporter genes are genes for resistance to antibiotics or herbicides, hydrolase genes, fluorescence protein genes, bioluminescence genes, sugar or nucleotide metabolism genes or biosynthesis genes such as the Ura3 gene, the Ilv2 gene, the luciferase gene, the ß-galactosidase gene, the gfp gene, the 2-desoxyglucose-6-phosphate phosphatase gene, the ß-glucuronidase gene, ß-lactamase gene, the neomycin phosphotransferase gene, the hygromycin phosphotransferase gene or the BASTA (= ~~gluphosinate [sic]~~ glufosinate resistance) gene. These genes make it possible easily to measure and quantify the transcriptional activity and thus gene expression. It is thus possible to identify sites in the genome which show differences in productivity.

Please replace the paragraph on page 19, at indicated lines 10 – 23 with the following paragraph:

Expression vectors used in prokaryotes frequently make use of inducible systems with and without fusion proteins or fusion oligopeptides, it being possible for these fusions to take place both at the N terminus and at the C terminus or other domains of a protein which can be used. As a rule, such fusion vectors are intended to: i.) increase the RNA expression rate, ii.) increase the protein synthesis rate which can be achieved, iii.) increase the solubility of a protein, or iv.) simplify purification by a binding sequence which can be used for affinity chromatography. Proteolytic cleavage sites are frequently also introduced by fusion proteins, ~~enabling elimination of part of the fusion protein also of the purification [sic]~~ enabling purification to include elimination of part of the fusion protein as well. Such recognition sequences for proteases recognize ~~are [sic]~~, for example, factor Xa, thrombin and enterokinase.

Please replace the paragraph on page 22, indicated line 30 – page 23, indicated line 2 with the following paragraph:

Depending on the choice of the promoter, expression of the Δ6-desaturase gene may take place specifically in the leaves, in the seeds, the tubers or other parts of the plant. The present invention furthermore relates to such transgenic plants which overproduce fatty acids, oils or lipids, and to their propagation material and their plant cells, tissue or plant parts. A preferred subject according to the invention is transgenic plants of, for example, crop plants such as maize, oats, rye, wheat, barley, ~~maize [sic]~~ rice, soybean, sugarbeet, canola, triticale, sunflower, flax, hemp, tobacco, tomato, coffee, cacao, tea, carrot, capsicum, oilseed rape, tapioca, carcaba, arrowroot, tagetes, alfalfa, lettuce and the various tree, nut and grapevine species, potatoes, in particular oil-containing crop plants such as soybean, peanut, castor, borrage, linseed, sunflower, canola, cotton, flax, oilseed rape, coconut, oilpalm, safflower (Carthamus tinctorius) or cacao bean, laboratory plants such as Arabidopsis, or other plants such as mosses or algae comprising a functional nucleic acid sequence according to the invention or a functional expression cassette. Functional in this context means that an enzymatically active enzyme is formed.

Please replace the paragraph on page 23, at indicated lines 4 – 12 with the following paragraph:

The expression cassette or the nucleic acid sequences according to the invention comprising a Δ6-desaturase gene sequence can additionally also be used for the transformation of the organisms which have been mentioned

above by way of example, such as bacteria, cyanobacteria, filamentous fungi, ciliates, animals or algae, with the aim of increasing the content in fatty acids, oils or lipids ~~[lacuna]~~ of Δ6-double bonds. Preferred transgenic organisms are bacteria, cyanobacteria, filamentous fungi or algae.

Please replace the paragraph on page 23, at indicated lines 14 – 31 with the following paragraph:

Transgenic organisms are to be understood as meaning organisms which comprise a foreign nucleic acid derived from another organism which encodes a Δ6-desaturase used in the process according to the invention. Transgenic organisms are also to be understood as meaning organisms which ~~comprises [sic]~~ comprise a nucleic acid which is derived from the same organism and encodes a Δ6-desaturase, this nucleic acid being present as an additional gene copy or not being present in the natural nucleic acid environment of the Δ6-desaturase gene. Transgenic organisms are also organisms in which the natural 3'- and/or 5'-region of the Δ6-desaturase gene has been modified over the initial organisms by targeted, recombinant modifications. Preferred transgenic organisms are those into which a foreign DNA has been introduced. Especially preferred are transgenic plants into which a foreign DNA has been introduced. Transgenic plants are to be understood as meaning individual plant cells and their cultures, such as, for example, callus cultures on solid media or in liquid culture, the plant parts and intact plants.

Please replace the paragraph on page 23, indicated line 33 – page 24, indicated line 5 with the following paragraph:

The invention furthermore relates to transgenic organisms

selected from the group of the plants, fungi, ciliates, algae, bacteria, cyanobacteria or animals, preferably transgenic plants or algae, comprising at least one isolated nucleic acid sequence encoding a polypeptide with Δ6-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence having the sequence shown in SEQ ID NO: 1,

b) nucleic acid sequences which, as a result of the degeneracy of the genetic code, are derived from ~~[lacuna]~~ the sequence shown in SEQ ID NO: 1,

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 1 which encode polypeptides with the amino acid sequences shown in SEQ ID NO: 2 and have at least 50% homology at the amino acid level without substantially reducing the enzymatic action of the polypeptides.

Please replace the paragraph on page 25, indicated line 43 – page 26, indicated line 5 with the following paragraph:

The lipids were extracted from the ~~S. [sic]~~ P. patens Protonema or from yeast cells using chloroform/methanol as described by Siebertz et al. (Eur. J. Biochem., 101, 1979: 429-438) and purified with diethyl ether by thin-layer chromatography (= TLC). The fatty acids obtained were transmethylated to give the corresponding methyl esters and analyzed by gas chromatography (= GC). The various methyl esters were identified using corresponding standards. Corresponding fatty acid pyrrolidides were obtained, and identified by GC-MS, as described by Anderson et al. (Lipids, 9, 1974: 185-190).

Please replace the paragraph on page 27, indicated line 32 – page 28, indicated line 1 with the following paragraph:

Eventually fragments of a Δ6-desaturase gene were cloned with the aid of a PCR reaction with the following degenerate oligonucleotides as primers:

A: TGGTGGAA(A/G)TGGA(C/A)ICA(T/C)AA and
B: GG(A/G)AA(A/C/G/T)A(A/G)(G/A)TG(G/A)TG(C/T)TC]

and the following temperature program:
94°C, 3 min; [94°C, 20 sec; 45°C, 30 sec; 72°C, 1 min], 30 cycles; 72°C, 5 min. For cloning, poly(A)RNA was isolated from 12-day-old P. patens Protonema ~~cultures [sic]~~ cultures. The above-described PCR was carried out with this poly(A)RNA. Fragments of the expected fragment length (500 to 600 bp) were cloned into pUC18 and sequenced. The deduced amino acid sequence of a PCR fragment showed similarities with known Δ6-desaturases. Since it was known that P. patens has a Δ6-desaturase, it was assumed that this clone encodes part of a Δ6-desaturase.

Please replace the paragraph on page 30, at indicated lines 13 – 19 with the following paragraph:

The reaction mixtures contained approximately ~~1 ng/micro l [sic]~~ 1 ng/μl template DNA, 0.5 μm of the oligonucleotides and, 200 μm deoxy-nucleotides (Pharmacia), 50 mM KCl, 10 mM Tris-HCl (pH 8.3 at 25°C, 1.5 mM $MgCl_2$) and 0.02 U/μl Pwo polymerase (Boehringer Mannheim) and are incubated in a Perkin Elmer PCR machine with the following temperature program:

Please replace the paragraph on page 30, indicated line 33 – page 31, indicated line 8 with the following paragraph:

For the transformation of plants, a further transformation vector based on pBin-USP was generated, and this transformation vector contains the Δ6-desaturase BaMHI fragment. pBin-USP is a derivative of plasmid pBin19. pBinUSP originated from pBin19, by inserting an USP promoter into pBin19 [Bevan et al. (1980) Nucl. Acids Res. 12, 8711] as ~~EcoRI-BaMHI [sic]~~ EcoRI-BamHI fragment. The polyadenylation signal is that of gene 3 of the T-DNA of the Ti-plasmid pTiACH5 (Gielen et al., (1984) EMBO J. 3, 835), where the nucleotides 11749-11939 were isolated as PvuII-HindIII fragment and, after the addition of SphI-linkers, cloned at the PvuII cleavage site between the SpHI-HindIII cleavage site of the vector. The USP promoter corresponds to the nucleotides 1-684 (Genbank Accession X56240), where part of the noncoding region of the USP gene was obtained in the promoter. The promoter fragment which is 684 base pairs in size was amplified with the aid of commercially available T7 standard primer (Stratagene) and with the aid of a synthesized primer via a PCR reaction using standard methods (primer sequence: 5'-GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGATCC GGATCTGCTGGCTATGAA-3'). The PCR fragment was subsequently cut with EcoRI/SalI and inserted into the vector pBin19 with OCS terminator. This gave rise to the plasmid named pBinUSP.

Please replace the paragraph on page 31, at indicated lines 13 – 20 with the following paragraph:

A construct using the v-ATPase-c1 promoter was generated analogously to the expression plasmid with the USP promoter. The promoter was cloned into the plasmid pBin19 with OCS terminator as an EcoRI/KpnI fragment and the P. patens Δ6-desaturase gene was inserted between promoter and terminator via BaMHI. The promoter corresponds to a ~~Beta [sic]~~ Beta vulgaris 1153 base pair fragment (Plant Mol Biol, 1999, 39:463-475).

Please replace the paragraph on page 32, at indicated lines 4 – 14 with the following paragraph:

Regenerated shoots were obtained on 2MS medium supplemented with kanamycin and Claforan, then, after rooting, transferred into soil and, after cultivation for two weeks, grown in a controlled-environment cabinet or in the greenhouse and allowed to flower, and mature seeds were harvested and analyzed for Δ6-desaturase expression by means of lipid analyses. Lines with increased contents of or ~~[sic]~~ double bonds at the Δ6 position were identified. In the stably transformed transgenic lines which functionally expressed the transgene, an increased content of double bonds at position Δ6 was found in comparison with untransformed control plants.

Please replace the paragraph on page 32, at indicated line 16 with the following paragraph:

Example ~~8 [sic]~~ 9: Lipid extraction from seeds